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Prevalence of 35delG and Met34Thr *GJB2* variants in Portuguese samples

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ABSTRACT

Objective: To estimate the prevalence of 35delG and Met34Thr variants in a Portuguese children's community sample and to compare these frequencies with nonsyndromic hearing-loss patients.
Methods: 502 children were randomly selected among the 8647 participants of the Portuguese birth cohort Generation XXI, and screened for Met34Thr and 35delG variants in the *GJB2* gene. These variants were also studied on 89 index-cases, observed in the Clinic of "Hereditary Hearing-loss" in Saint John's Hospital Center, presenting a mild to profound nonsyndromic hearing-loss.
Results: Among the 502 children from Generation XXI, 10 were heterozygous for the 35delG variant (95% Confidence Interval 1.03–3.68) and 1 homozygous (95% Confidence Interval 0.01–1.24). Other 10 children presented heterozygosity for the Met34Thr variant (95% Confidence Interval 1.03–3.68). No homozygous for the Met34Thr or compound heterozygotes (35delG/Met34Thr) were found. In the total of 89 nonsyndromic hearing-loss patients, 5 (95% Confidence Interval 2.11–12.8) were heterozygous and 7 (95% Confidence Interval 3.61–15.6) were homozygous for the 35delG variant. The Met34Thr variant was found in 4 patients, 2 heterozygous (95% Confidence Interval 0.13–8.31) and 2 homozygous (95% Confidence Interval 0.13–8.31).
Conclusion: The carrier frequency of 35delG and Met34Thr variants in a Portuguese sample was 1 in 50. Our data suggests that the 35delG mutation has an association with deafness. For the Met34Thr variant, no association was observed. However, Met34Thr seemed to conform to an additive model in hearing-loss.

1. Introduction

Hearing-loss (HL) is a very common congenital sensory impairment, affecting approximately 1 in 500 to 1000 newborns [1]. HL can be syndromic (30%) or nonsyndromic (70%) [2]. Nonsyndromic hearing-loss (NSHL) can be inherited in an autosomal dominant trait in 15–20% of the cases, in an autosomal recessive trait (80%), or also X-linked (2–3%) or mitochondrial (1%) [2]. More than 50% of cases of autosomal recessive NSHL in several world populations are attributed to mutations in the *GJB2* gene [3]. *GJB2* encodes gap junction protein connexin 26 (Cx26), which has been implicated in the maintenance of K^+ homeostasis in the inner ear [4].

1.1. Molecular genetics

The most common mutation in the *GJB2* gene is a deletion of a single guanine in a track of six guanines known as 35delG [2,5]. It is

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also the most common *GJB2* mutation causing NSHL in Portugal, with a prevalence of 15.9% [6].

Overall, in the European general population, a high frequency of carriers of the 35delG variant was also detected, with a frequency of 1 in 51 (1.96%) [7]. For many years, it was established that the highest carrier frequencies of 35delG in Europe were found in countries close to the Mediterranean (1 in 31; 3.2%) [5,8], but recent studies demonstrated that the highest carrier rates may actually be found in Belarus (1 in 17; 5.8%) and Estonia (1 in 22; 4.5%) [3,9]. To our knowledge, there is only one study estimating the carrier frequency of 35delG in 178 individuals from a Portuguese population in about 1 in 45 (2.2%) [8].

Another variation in the *GJB2* gene that has been found with a high prevalence in studies in NSHL populations, including Portugal, c.101 T → C (Met34Thr), a methionine-to-threonine substitution at amino acid 34 [2,6,10]. This variant was found at a lower frequency in NSHL patients, compared to the 35delG mutation [11]. However, in the general Caucasian population, several studies have found a higher frequency of the Met34Thr variant [12,13]. A carrier rate of approximately 1 in 37 (2.69%) for the Met34Thr variation and approximately 1 in 73 (1.36%) for the 35delG mutation was found in a United Kingdom (UK) study [14]. To our knowledge, no published studies in the Portuguese population have estimated the carrier rate of this variant, but studies in Europe demonstrated very different carrier frequencies among countries, about 1 in 25 (4%) in Britain/Ireland and Finland, 1 in 43 (2.3%) in France and 1 in 17 (5.8%) in Estonia [9,13,15].

1.2. Clinical genetics

The degree of NSHL in 35delG homozygotes has already been widely documented by several studies, stating that these individuals usually present moderate to profound NSHL [10,16]. In relation to the 35delG heterozygotes, there are contradictory results in the literature [1,7,14]. Recent studies showed statistically significant differences between carriers' audiograms and non-carriers' only at extra-high frequencies [1,14], despite previous studies having described differences at 6000–8000 Hz. The findings in otoacoustic emissions are non-conclusive in the literature [1,17,18].

The role of the Met34Thr variant in NSHL is much less understood and the data available is controversial. Met34Thr homozygotes or in compound heterozygosity with a known pathogenic mutation on the other allele usually result in mild to moderate NSHL [9,15,19]. A UK study found that carriers of the Met34Thr variant presented a decrease at extra-high frequency thresholds [14]. There are some reports of clinical findings in Met34Thr carriers, therefore, this subject still remains controversial [11,14,20–22].

The purpose of this study was to estimate the prevalence of 35delG and Met34Thr variants in a Portuguese children's community sample and to compare these frequencies with nonsyndromic hearing-loss patients.

2. Participants and methods

2.1. Participants

2.1.1. Community participants

Generation XXI is a birth cohort of 8647 newborns recruited in 2005–2006 in the Porto Metropolitan Area, North of Portugal. The recruitment occurred at all public maternity units, responsible for 95% of all births in the region, as described in a previous study [23]. Four years after birth, the whole cohort was invited to a follow-up evaluation. For the present study, 545 of the participants attending the follow-up evaluation were randomly selected, in order to study the carrier rate of the most common mutation in the *GJB2* gene in a Portuguese population—35delG. The sample size was calculated based on the estimated frequency of the 35delG mutation in Mediterranean populations—1 in 31 [5], with a level of significance of 5% and a power of 80%. The inclusion criterion was having at least three generations of Caucasian Portuguese parental ancestry. One of the most controversial variants associated to NSHL, Met34Thr, was also studied as a secondary aim. Of the 545 selected participants, only 502 children were included in the study, due to constraints regarding sample quality, which did not allow us to get DNA amplification by PCR (*polymerase chain reaction*).

2.1.2. Patients

Eighty-nine consecutive patients that attended the Clinic of "Hereditary Hearing-loss" in Saint John's Hospital Center, from June 2011 untill December 2014, with mild to profound NSHL, from the North Region of Portugal, were invited to participate in the study. Audiological evaluation was performed according to the GenDeaf study group recommendations, using auditory brainstem response tests (ABR) or pure tone audiometry (PTA) [24]. Syndromic hearing-loss and causes of acquired deafness were excluded.

2.1.3. Ethics

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (World Medical Association, 2013). The study was approved by the University of Porto's Medical School/Saint Jonh's Hospital Center ethics committee and a signed informed consent according to the Declaration of Helsinki was obtained of all participants.

2.2. Methods

2.2.1. Genetic analysis

DNA samples of oral swabs were amplified by PCR (*polymerase chain reaction*), using the following forward (Cx26F: 5′-TCTTTTCCA-GAGCAAACCGCC-3′) and reverse (Cx26R: 5′-TGAGCACGGGTTGCCT-CATC-3′) specific primers for the *GJB2* gene exon 2. The enzyme used was Type-it Microsatellite PCR kit from Qiagen. The cycling profile consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 48 s, 63 °C for 48 s, 72 °C for 1 min and a final extension step at 72 °C for 30 min. PCR products purification using AmpureXP® was performed to remove the contaminants. The sequencing samples were purchased as an outsourced service by Primbio Research Institute, Exton, USA. The analysis of products was performed on an automated sequencer (Applied Biosystems® 3730xl DNA Analyzer) and the results were analyzed with Applied Biosystems® Sequencing Analysis v.5.4 software. All variants found were then confirmed in our department (ABI Prism 3500®). For those with the 35delG mutation, the sequencing reaction was repeated using the reverse primer in order to exclude the Met34Thr variant.

2.2.2. Statistical analysis

The statistical analyses were performed using SPSS Statistics 22®, GraphPad® and Epi Info® softwares. The Chi-square test was used to determine whether the allelic frequencies are in Hardy–Weinberg equilibrium and to test for linkage disequilibrium between the two minor variants. As expected values in some cells of the contingency table were below 5, the p level used was obtained from the Fisher's exact test. Differences in the carrier frequencies between the two groups were also tested by the Chi-square test. The Chi-square test for trend was used to assess for a possible dosage effect of the 35delG and Met34Thr variants upon the association of hearing-loss. This Chi-square test for trend assumed an additive genetic model, that is, the risk of a disease conferred by an allele is increased additively [25,26].

Table 1
Results of 35delG and Met34Thr variants in NSHL patients and Generation XXI children. CI—confidence interval; *n*/total—number of children with that characteristic in the total of individuals.

		Normal *n*/total (%) (95% CI)	Heterozygote *n*/total (%) (95% CI)	Homozygote *n*/total (%) (95% CI)
35delG	NSHL patients	**77/89** (86.50) (77.74–92.27%)	**5/89** (5.60) (2.11–12.80%)	**7/89** (7.90) (3.61–15.60%)
	Generation XXI	**491/502** (97.80) (96.07–98.82%)	**10/502** (2.00) (1.03–3.68%)	**1/502** (0.20) (0.01–1.24%)
Met34Thr	NSHL patients	**85/89** (95.50) (88.65–98.59%)	**2/89** (2.20) (0.13–8.31%)	**2/89** (2.20) (0.13–8.31%)
	Generation XXI	**492/502** (98.00) (96.32–98.95%)	**10/502** (2.00) (1.03–3.68%)	**0/502** (0.00) (0.00–0.92%)

3. Results

3.1. Frequency of 35delG and Met34Thr GJB2 variants in a Portuguese sample

A total of 502 children from the Generation XXI cohort were studied for the presence of the 35delG and Met34Thr variants in the *GJB2* gene. The results obtained and carrier rates are summarized in Table 1. The 35delG mutation was found in 11 children, 10 heterozygotes (35delG/wild-type) and 1 homozygote (35delG/35delG), indicating a carrier rate of 1 in 50 (95% Confidence Interval (CI) 1.03–3.68%). The case with homozygosity for 35delG/35delG was subsequently confirmed to have bilateral severe NSHL, through self-reported data collected during the follow-up evaluation of Generation XXI. None of the other participants presented a phenotype similar to this one. Ten children presented heterozygosity for the Met34Thr variant, also indicating a carrier rate of 1 in 50. No homozygous for Met34Thr or compound heterozygotes (35delG/Met34Thr) were found in this study.

3.2. Frequency of 35delG and Met34Thr GJB2 variants in patients with NSHL

Eighty-nine individuals with mild to profound NSHL were studied for the presence of 35delG and Met34Thr variants in the *GJB2* gene. The results obtained and carrier rates are represented in Table 1. The 35delG mutation was found in 12 patients, 5 heterozygotes (35delG/wild-type) and 7 homozygotes (35delG/35delG), indicating a carrier rate of approximately 1 in 18 (95% CI 2.11–12.80%). Four individuals presented the Met34Thr variant, 2 in heterozygosity (Met34Thr/wild-type) and 2 in homozygosity (Met34Thr/Met34Thr), estimating a carrier rate of approximately 1 in 44 (95% CI 0.13–8.31%). No compound heterozygosity was found among the analyzed cases.

3.3. Comparison between the community participants and NSHL patients

The analysis of the Hardy–Weinberg equilibrium (HWE) of the genotypes within the Generation XXI cohort with a Chi-square test showed evidence that this population is in HWE ($p = 0.975$ and $p = 0.998$ for 35delG and Met34Thr alleles, respectively). There was no evidence of linkage disequilibrium among the alleles ($p = 0.910$) the Chi-square test for trend showed statistically significant dosage effects of both the 35delG mutation ($p = 0.001$) and the Met34Thr variant ($p = 0.050$).

We also compared the carrier frequencies found in the NSHL patients with those in children from Generation XXI. The differences in the carrier frequencies between the two groups were statistically significant for the 35delG mutation ($p = 0.001$), but not for the Met34Thr variant ($p = 0.245$).

4. Discussion

In this study, a group of 502 children from the Generation XXI cohort and a group of 89 patients with mild to profound NSHL were screened for the 35delG and Met34Thr *GJB2* variants. Results presented here confirm that these variants are frequent in the general population. In NSHL patients, a lower carrier frequency of the Met34Thr variant (1 in 44) compared to 35delG (1 in 18) was found, as previously described in a study from Snoeckx et al. [11]. In Generation XXI, similar carrier rates were found for the two variants (1 in 50).

Although statistically significant differences were found in the 35delG carrier frequencies ($p = 0.001$) for NSHL and Generation XXI groups, the carrier frequencies of the Met34Thr variant were not statistically different ($p = 0.245$). If this variant was unequivocally associated with deafness, we would expect a statistically significant higher number of individuals with this variant in the NSHL group compared to the general population. However, the frequencies were similar (2.2% vs. 2.0%, respectively). The absence of statistically significant differences could be the result of ascertainment bias toward more severe NSHL, as persons with milder NSHL are less likely to request an audiological assessment [11] or could result from low statistical power to find association between the variant and hearing-loss in our group of patients, due to a low number of patients studied. It would be necessary to study this variant in a larger number of patients with NSHL to better characterize this potential association. However, the results of the Chi-square test for trend suggest that the risk of hearing-loss related to the Met34Thr variant may conform to an additive model ($p = 0.050$), indicating that the Met34Thr variant may be a risk factor for NSHL, directly related to this variant or acting as a modifying allele, as previously described [27]. For the 35delG mutation, the statistical evidence in favor of an additive pathogenic effect is much more robust ($p < 0.001$).

The carrier rate of the 35delG variant found in the Portuguese population cohort (1 in 50) was lower than the one estimated for Portugal in the Gasparini et al. study (1 in 45) and for other Mediterranean countries (1 in 35) [8,28]. However, this value is similar to the average carrier rate of 35delG *GJB2* variants in Europe (1 in 51) [8].

To our knowledge, this is the first study estimating the carrier rate of the Met34Thr variant in the Portuguese population. The carrier rate obtained in this study (1 in 50) is lower than rates found in other European countries (1 in 17 in Estonia, 1 in 37 in UK and 1 in 43 in France) [13]. Genetic studies of the *GJB2* gene showed that the Met34Thr variant is more prevalent in mid-west America (0.765%), United Kingdom and Ireland (1.984%), but has a reduced frequency or even absence in France, Spain, Italy and individuals of Japanese ethnic origin [15]. Houseman et al. suggested that these differences in populations' carrier frequencies could be explained by a single ancestral mutation occurring originally in the United Kingdom or Ireland [15].

The effect of the Met34Thr variant on NSHL still remains controversial. An autosomal dominant trait was first described to the Met34Thr variant [10]. Nevertheless, a second mutation in the *GJB2* gene has been identified, which is correlated with hearing-loss, causing doubts about this autosomal dominant trait [22,27]. Subsequently, an autosomal recessive form of NSHL has been suggested [15] and then, as several authors had reported normal hearing in heterozygous carriers of the Met34Thr variant

associated with other *GJB2* recessive variants, it was considered not pathogenic [29–32]. These authors hypothesized that the frequent presence of Met34Thr patients in NSHL patients could be due to the high carrier rate of this variant in the general population [9,32]. More recently, studies combining genetic, clinical, biochemical, electrophysiological properties and structural modeling studies supported the hypothesis that Met34Thr is a pathological variant associated with hearing impairment by showing that, at a cellular level, despite the Cx26-Met34Thr protein being correctly synthesized and targeted to the plasma membrane of HeLA cells, it inefficiently forms intercellular channels that display abnormal electrical activity and retain only 11% of the unitary conductance of Cx26-wild-type [22,33]. The results of the additive model study found in this work seem to corroborate this finding. A recent work from our group showed evidence that this variant might correlate with a dominant hearing-loss with incomplete penetrance and a variable expression of the phenotype [34].

5. Conclusion

A high carrier frequency of the 35delG and Met34Thr *GJB2* variants (1 in 50) was found in Generation XXI participants. Our data suggests that the 35delG mutation has a clear association with NSHL. Although no statistically significant differences were found between the carrier frequencies in the two groups for Met34Thr, it seemed to conform to an additive model in hearing-loss.

Conflict of interest statement

None.

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References

[1] D. Groh, P. Seeman, M. Jilek, J. Popelar, Z. Kabelka, J. Syka, Hearing function in heterozygous carriers of a pathogenic GJB2 gene mutation, Physiol. Res. 62 (2013) 323–330.
[2] C. Nogueira, M. Coutinho, C. Pereira, A. Tessa, F.M. Santorelli, L. Vilarinho, Molecular investigation of pediatric Portuguese patients with sensorineural hearing loss, Genet. Res. Int. 2011 (2011) 587602.
[3] N. Danilenko, E. Merkulava, M. Siniauskaya, O. Olejnik, A. Levaya-Smaliak, A. Kushniarevich, et al., Spectrum of genetic changes in patients with non-syndromic hearing impairment and extremely high carrier frequency of 35delG GJB2 mutation in Belarus, PLoS ONE 7 (2012) e36354.
[4] E. Baysal, Y.A. Bayazit, S. Ceylaner, N. Alatas, B. Donmez, G. Ceylaner, et al., GJB2 and mitochondrial A1555G gene mutations in nonsyndromic profound hearing loss and carrier frequencies in healthy individuals, J. Genet. 87 (2008) 53–57.
[5] G. Lucotte, High prevalences of carriers of the 35delG mutation of connexin 26 in the Mediterranean area, Int. J. Pediatr. Otorhinolaryngol. 71 (2007) 741–746.
[6] T.D. Matos, H. Simoes-Teixeira, H. Caria, A.C. Goncalves, J. Chora, Mdo Cea Correia, Spectrum and frequency of GJB2 mutations in a cohort of 264 Portuguese nonsyndromic sensorineural hearing loss patients, Int. J. Audiol. 52 (2013) 466–471.
[7] A. Franze, A. Caravelli, F. Di Leva, E. Marciano, G. Auletta, F. D'Aulos, et al., Audiometric evaluation of carriers of the connexin 26 mutation 35delG, Eur. Arch. Otorhinolaryngol. 262 (2005) 921–924.
[8] P. Gasparini, R. Rabionet, G. Barbujani, S. Melchionda, M. Petersen, K. Brondum-Nielsen, et al., High carrier frequency of the 35delG deafness mutation in European populations. Genetic analysis consortium of GJB2 35delG, Eur. J. Hum. Genet. 8 (2000) 19–23.
[9] R. Teek, K. Kruustuk, R. Zordania, K. Joost, T. Reimand, T. Mols, et al., Prevalence of c.35delG and p.M34T mutations in the GJB2 gene in Estonia, Int. J. Pediatr. Otorhinolaryngol. 74 (2010) 1007–1012.
[10] D.P. Kelsell, J. Dunlop, H.P. Stevens, N.J. Lench, J.N. Liang, G. Parry, et al., Connexin 26 mutations in hereditary non-syndromic sensorineural deafness, Nature 387 (1997) 80–83.
[11] R.L. Snoeckx, P.L. Huygen, D. Feldmann, S. Marlin, F. Denoyelle, J. Waligora, et al., GJB2 mutations and degree of hearing loss: a multicenter study, Am. J. Hum. Genet. 77 (2005) 945–957.
[12] G.E. Green, D.A. Scott, J.M. McDonald, G.G. Woodworth, V.C. Sheffield, R.J. Smith, Carrier rates in the midwestern United States for GJB2 mutations causing inherited deafness, JAMA 281 (1999) 2211–2216.
[13] A.F. Roux, N. Pallares-Ruiz, A. Vielle, V. Faugere, C. Templin, D. Leprevost, et al., Molecular epidemiology of DFNB1 deafness in France, BMC Med. Genet. 5 (2004) 5.
[14] A. Hall, M. Pembrey, M. Lutman, C. Steer, M. Bitner-Glindzicz, Prevalence and audiological features in carriers of GJB2 mutations, c.35delG and c.101T>C (p.M34T), in a UK population study, BMJ Open 2 (4) (2012) 3–8.
[15] M.J. Houseman, L.A. Ellis, A. Pagnamenta, W.L. Di, S. Rickard, A.H. Osborn, et al., Genetic analysis of the connexin-26 M34T variant: identification of genotype M34T/M34T segregating with mild-moderate non-syndromic sensorineural hearing loss, J. Med. Genet. 38 (2001) 20–25.
[16] A. Murgia, E. Orzan, R. Polli, M. Martella, C. Vinanzi, E. Leonardi, et al., Cx26 deafness: mutation analysis and clinical variability, J. Med. Genet. 36 (1999) 829–832.
[17] B. Engel-Yeger, S. Zaaroura, J. Zlotogora, S. Shalev, Y. Hujeirat, M. Carrasquillo, et al., The effects of a connexin 26 mutation-35delG—on oto-acoustic emissions and brainstem evoked potentials: homozygotes and carriers, Hear Res. 163 (2002) 93–100.
[18] L.S. Silva, R.C. Netto, S.G. Sanches, R.M. Carvallo, Auditory measurements in parents of individuals with autosomal recessive hearing loss, Pro-Fono 22 (2010) 403–408.
[19] R.A. Cucci, S. Prasad, P.M. Kelley, G.E. Green, K. Storm, S. Willocx, et al., The M34T allele variant of connexin 26, Genet. Test. 4 (2000) 335–344.
[20] A. Pollak, A. Skorka, M. Mueller-Malesinska, G. Kostrzewa, B. Kisiel, J. Waligora, et al., M34T and V37I mutations in GJB2 associated hearing impairment: evidence for pathogenicity and reduced penetrance, Am. J. Med. Genet., A 143A (2007) 2534–2543.
[21] M. Coco, F. Salvinelli, F. Greco, M. Trivelli, L. D'Emidio, A. Mesoraca, et al., Significance of heterozygosis M34T mutation of GJB2 gene in non-syndromic congenital deafness. Retrospective analysis of 12,472 samples of amniotic fluid, J. Prenat. Med. 7 (2013) 56–58.
[22] M. Bicego, M. Beltramello, S. Melchionda, M. Carella, V. Piazza, L. Zelante, et al., Pathogenetic role of the deafness-related M34T mutation of Cx26, Hum. Mol. Genet. 15 (2006) 2569–2587.
[23] P.S. Larsen, M. Kamper-Jorgensen, A. Adamson, H. Barros, J.P. Bonde, S. Brescianini, et al., Pregnancy and birth cohort resources in Europe: a large opportunity for aetiological child health research, Paediatr. Perinat. Epidemiol. 27 (2013) 393–414.
[24] M.V.C.G. Mazzoli, V. Newton, M. Giarbini, F. Declau, A. Parving, GenDeaf Study Group, Recommendations for the description of genetic and audiological data for families with nonsyndromic hereditary hearing impairment, Audiol. Med. 1 (2003) 148–150.
[25] C.M. Lewis, Genetic association studies: design, analysis and interpretation, Brief Bioinform. 3 (2002) 146–153.
[26] C. Minelli, J.R. Thompson, K.R. Abrams, A. Thakkinstian, J. Attia, The choice of a genetic model in the meta-analysis of molecular association studies, Int. J. Epidemiol. 34 (2005) 1319–1328.
[27] D.P. Kelsell, A.L. Wilgoss, G. Richard, H.P. Stevens, C.S. Munro, I.M. Leigh, Connexin mutations associated with palmoplantar keratoderma and profound deafness in a single family, Eur. J. Hum. Genet. 8 (2000) 469–472.
[28] L. Van Laer, P. Coucke, R.F. Mueller, G. Caethoven, K. Flothmann, S.D. Prasad, et al., A common founder for the 35delG GJB2 gene mutation in connexin 26 hearing impairment, J. Med. Genet. 38 (2001) 515–518.
[29] F. Denoyelle, D. Weil, M.A. Maw, S.A. Wilcox, N.J. Lench, D.R. Allen-Powell, et al., Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene, Hum. Mol. Genet. 6 (1997) 2173–2177.
[30] P.M. Kelley, D.J. Harris, B.C. Comer, J.W. Askew, T. Fowler, S.D. Smith, et al., Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss, Am. J. Hum. Genet. 62 (1998) 792–799.
[31] D.A. Scott, M.L. Kraft, R. Carmi, A. Ramesh, K. Elbedour, Y. Yairi, et al., Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss, Hum. Mutat. 11 (1998) 387–394.
[32] D. Feldmann, F. Denoyelle, N. Loundon, D. Weil, E.N. Garabedian, R. Couderc, et al., Clinical evidence of the nonpathogenic nature of the M34T variant in the connexin 26 gene, Eur. J. Hum. Genet. 12 (2004) 279–284.
[33] F. Zonta, D. Buratto, C. Cassini, M. Bortolozzi, F. Mammano, Molecular dynamics simulations highlight structural and functional alterations in deafness-related M34T mutation of connexin 26, Front. Physiol. 5 (2014) 85.
[34] M. Dória, S. Fernandes, C.P. Moura, Study of Met34Thr variant in nonsyndromic hearing loss in four Portuguese families, Arq. Med. (2015) (in press).